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Comparison of Photosynthetic Pigment Contents of the Resurrection Plants *Ramonda serbica* and *Ramonda nathaliae* of Some Different Populations from Kosovo, Albania and Macedonia

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ABSTRACT

The resurrection plants *Ramonda serbica* and *Ramonda nathaliae* are the physiological model plant and endemic species from Balkan Peninsula. Study was carried out to assess the impact of different populations on photosynthetic pigment contents and their effect on physiological activity of these species. The experiment was conducted with leaves of *R. serbica* collected from nine populations in Kosovo, three in Albania and two in Macedonia, while for *R. nathaliae* four populations in Macedonia. For this purpose plants after collection from their natural populations were kept for 24 hours at constant temperature and humidity and in natural photoperiod. According to our data the pigment contents (Total chl + Carot) of *R. serbica*, measured as mg per gram dry weight (DW), were higher (7.06 $mg \cdot g^{-1}$ DW) in Radaci populations (Kosovo) and lower (4.63 $mg \cdot g^{-1}$ DW) in Jukniu Mountain-Kruja (Albania). On the other hand, the higher (5.28 $mg \cdot g^{-1}$ DW) of pigment contents (Total chl + Carot) of *R. nathaliae* were observed in Vorca populations and the lower (4.24 $mg \cdot g^{-1}$ DW) in Kaparllëk populations. Ratio chlorophyll a/b of *R. serbica* in Kruja Castle populations from Albania was the higher (3.68) comparing with Zhlebi populations from Kosovo (1.68). Similarly in case of *R. nathaliae* the higher (3.36) value was in Kaparllëk populations and the lower (3.12) in Matka populations. The data obtained were further analyzed using one-way ANOVA and a significant change was recorded in the different populations. These studies clearly indicate that the *Ramonda* plants from different ecological habitats there have been changes of photosynthetic pigment contents.

Keywords: Resurrection Plants; Physiological Model; Populations; Photosynthetic Pigments

1. Introduction

A small number of higher plant species, not closely related, have adapted to environments with rapidly developing and often extended periods of extreme dryness, followed by sudden water availability. These are called desiccation-tolerant or resurrection plants. Examples are *Ramonda serbica* and *Ramonda nathaliae*, a rare resurrection plant of the Balkan Peninsula, an endemic relicts of the tertiary period.

The species of family *Gesneriaceae* are good examples of ecophysiological divergence regarding the plant water relations and the adaptations to the conditions of water regime in the habitat. Under the conditions of water deficit in the habitat, these plants gradually wilt and pass to anabiosis. Resurrection plants are usually subdivided into homoiochlorophyllous plants retaining their chlorophyll (Chl) during desiccation and poikilochlorophyllous plants where desiccation results in the loss of Chl which must be re-synthesized following rehydration [1]. However, pigment loss and destruction of the other thylakoid pigments are highly organised responses to desiccation, realised via a well-defined metabolic pathway [1]. From an ecological point of view, *R. serbica* is a perennial, herbaceous, shade-adapted species belonging to the group of homoiochlorophyllous poikilohydric plants which preserve more than 80% of the chlorophyll content during dehydration [2,3].

Its ability to survive in harsh environmental conditions has so far been studied in terms of morphologic, physicologic, and biochemical as well as the propagation through *in vitro* cultivation. This research has brought the evidence of adaptive features necessary to preserve cell membrane integrity [4], antioxidative capacity [5,6], photosynthetic activity [1], CO_2 fixation and chlorophyll *a* fluorescence [7], genome size variation and polyploidy [8], osmotic adjustment [9], seed germination [10] and *in vitro* cultivation from seeds of *Ramonda* plants [11-13].

Nevertheless, up to now there is only a few data from other authors for photosynthetic pigments content of *Ramonda serbica* or similar species from *Geseneriaceae* in different populations. Right now still are missing the study belonging to *Ramonda* species on concentration of photosynthetic pigments.

We are incited to find the concentration of chlorophyll a, b, total chlorophyll, carotenoids and their ratio from different ecological habitats conditions and their impact on ecophysiological activity for these plants.

2. Materials and Methods

The resurrection plants *Ramonda serbica* and *Ramonda nathaliae* were collected from their natural habitat. *R. serbica* in nine populations in Kosovo, three in Albania and two in Macedonia, while for *R. nathaliae* four populations in Macedonia (**Table 1**). Plants of the same age were harvested together with the layer of soil on which they grew.

For the photosynthetic pigments content study, three leaves of similar ages, comparable in size, were collected from five plants from the middle of the rosette.

2.1. Chlorophyll (*a*, *b* and Total) and Carotenoids Analysis

Pigments were extracted by grinding 60 - 80 mg freshly sampled leaves in 80% (v/v) acetone/water containing $MgCO_3$ (0.5% w/v) at room temperature for 24 h in the dark. Photosynthetic pigments of all the samples were extracted in triplicate to minimize experimental errors. Concentration of chlorophyll and carotenoid contents were measured by using absorbance recorded at 663 nm, 644 nm and 452.5 nm for maximum absorption of chlorophyll "*a*" (Chl *a*), chlorophyll "*b*" (Chl *b*) and Carotenoids, respectively. The extinction coefficients were determined by a UV-Vis spectrophotometer. Pigment contents were calculated in $mg \cdot g^{-1}$ dry leaf weight (DW) by applying the absorption coefficient equations described

Table 1. Geographic origin and description of *Ramonda* plants.

Code of populations	Species	Locality of populations	Directions	Altitude (m)	Country
1	*Ramonda nathaliae*	Gorge of river Matka	N	348	Macedonia
2	*Ramonda nathaliae*	Kaparllëk	N and NE	448	Macedonia
3	*Ramonda nathaliae*	Cerovë	N and NE	591	Macedonia
4	*Ramonda nathaliae*	Vorcë	N and NE	407	Macedonia
5	*Ramonda serbica*	Gorge of River Radika	N	920	Macedonia
6	*Ramonda serbica*	Llukovo	N	630	Macedonia
7	*Ramonda serbica*	Gorge of Zhlebi	N and NE	1250	Kosovo
8	*Ramonda serbica*	Radac	N	545	Kosovo
9	*Ramonda serbica*	Gorge river of Sushica	N	676	Kosovo
10	*Ramonda serbica*	Canyon of Rugova	N	800	Kosovo
11	*Ramonda serbica*	Gorge of Koprivnik	N	750	Kosovo
12	*Ramonda serbica*	Gorge of River Prizren	N	530	Kosovo
13	*Ramonda serbica*	Gorge of Rusenica	N	1340	Kosovo
14	*Ramonda serbica*	Gorge of Matosi	N	910	Kosovo
15	*Ramonda serbica*	Shkëmbi i përgjakur	N	1170	Kosovo
16	*Ramonda serbica*	Kruja Castle	N and NE	565	Albania
17	*Ramonda serbica*	Jukniu Mountain	N and NE	827	Albania
18	*Ramonda serbica*	Petrela Castle	N	340	Albania

by Lichtenthaler (1986) [14]:

$$\text{Chl } a \ \left(\text{mg}\cdot\text{g}^{-1}\ \text{DW}\right) = \left[10.3\left(\text{OD663}\right) - 0.918\left(\text{OD644}\right)\right] \times \text{V} \times 100/\text{FW} \times \text{DW}.$$

$$\text{Chl } b \ \left(\text{mg}\cdot\text{g}^{-1}\ \text{DW}\right) = \left[19.7\left(\text{OD644}\right) - 3.87\left(\text{OD663}\right)\right] \times \text{V} \times 100/\text{FW} \times \text{DW}.$$

$$\text{Carotenoids}\left(\text{mg}\cdot\text{g}^{-1}\ \text{DW}\right) = \left[4.75\left(\text{OD452.5}\right) - 0.226\left(\text{Chl } a + \text{Chl } b\right)\right] \times \text{V} \times 100/\text{FW} \times \text{DW}.$$

where

DW = Dry leaf weight.
FW = Fresh leaf weight.
OD = Optical density.
V = Volume of sample.

2.2. Data Analysis

The experiment was performed in a randomized design with five replicates. Differences among parameters and between the populations were tested using SPSS 17 statistical program. Statistical variance analysis of the all data was performed using one-way ANOVA and compared with least significant difference (LSD) at the 5% and 1% level. Mean comparison was performed with Duncan's test at the 5% level of significance.

3. Results and Discussions

Based on the obtained results and presented on the **Tables 2** and **3**, high significant differences at $P < 0.01$ have been ascertained between populations, for all researched parameters of *Ramondas* plants on different populations. The effect of different ecogeographic factors combine on highest differences on photosynthetic pigment concentration and their ratio between different populations.

At *R. nathaliae*, the highest content of Chl *a*, Caretenoids and Total Chlorophyll + Carotenoids has been ascertained at Vorca population, whereas the content of Chl *b* and Total Chl was higher at Gorge of river Matka population. The lowest content of all photosynthetic pigments was obtained at Kaparllëk population (**Table 2**). At Gorge of river Matka population, the Ratio Chl *a/b* (**Figure 1**) was the lowest (3.12), whereas the Ratio Total Chl/Carot was the highest (4.53) compared to other populations. Based on the results collected in the field, the Gorge of River Matka population had less sunlight due to the hindrance by the forest trees. For this reason the Chl *a*/*b* ratio has been frequently used as an indicator of plant response to shading [15].

Based on the gained and analysed results with One-way ANOVA and compared with Duncan test, at *R. serbica* leaves, the highest photosynthetic pigments content (**Table 3**) was ascertained at Radac and Llukovo populations. The similar results were gained for the Gorge of Matosi population, too. Out of all investigated populations, it is worth mentioning that these three populations have been with less sunlight compared with other populations. This increase of the photosynthetic pigments content is justified by the fact which for many plant species that grow under environmental shade conditions will result in growth of pigment content. In addition, Gonçalves *et al*. (2001) [16] at Mahogany and Tonka bean, leaf chlorophyll concentrations (Chl *a*, Chl *b*, Chl *tot*) on a fresh mass basis were higher in shade leaves than in sun leaves. According to Tan *et al*. (2000) [17] there is widespread interest in chlorophylls and their degradation reactions. Chlorophyll is synthesized and degraded (photo oxidation) under irradiation. At high irradiance, however, the degradation rate overtakes the rate of synthesis, therefore, a lower chlorophyll concentration is observed. Due to this fact, shade leaves in comparison with sun leaves tend to show higher chlorophyll concentrations per unit leaf weight [18].

Lower photosynthetic pigments content was observed at *R. serbica* population from Albania, especially in Jukniu Mountain and Kruja Castle populations. These populations are with more intensity of the sunlight compared with other populations from Albania, Kosovo and Macedonia (**Table 3**). Based on statistical analysis results with Duncan test, the populations' alignment with approximate content of photosynthetic pigments clearly indicates the similar ecological conditions of these populations.

The highest content of Chl *a*, Total Chl and Total Chl/Carot (**Table 3**) for all the investigated population of *R. serbica* was ascertained at Radac population (4.47, 5.90, and 7.06 $\text{mg}\cdot\text{g}^{-1}$ DW, respectively), whereas the lowest at Jukniu Mountain (2.86, 3.70, and 4.63 $\text{mg}\cdot\text{g}^{-1}$ DW, respectively). For the Chl *b* the highest content was at Gorge of Zhlebi population (1.76 $\text{mg}\cdot\text{g}^{-1}$ DW), whereas the lowest at Shkembi *i* pergjakur population (1.42 $\text{mg}\cdot\text{g}^{-1}$ DW). Our results of higher Chl *b* in the shade environment are in accordance with other authors results. Gonçalves *et al*. (2001) [16] showed that Chl *b* concentration on a mass basis of tonka bean and mahogany was higher in the shade environment.

The highest Carotenoids concentrations was at Gorge of Matosi population (1.26 $\text{mg}\cdot\text{g}^{-1}$ DW), whereas the lowest at Gorge of Zhlebi population (0.81 $\text{mg}\cdot\text{g}^{-1}$ DW). Another very important indicator for the intensity of sunlight is also Ratio Chl *a/b*, where the highest values for this parameters (**Figure 1**) were shown at Kruja Castle and Jukniu Mountain populations (3.68 and 3.42, re-

Table 2. The effects of different populations of *R. nathaliae* from Macedonia on photosynthetic pigment content in mg per gram dry weight (DW).

Code of populations		Chl *a*	Chl *b*	Total Chl	Carotenoids	Total Chl + Carot
		Mean ± SX	Mean ± SX	Mean ± SX	Mean ± SX	Mean ± SX
1		3.27 ± 0.16 a	1.06 ± 0.06 a	4.33 ± 0.22 a	0.95 ± 0.04 b	5.28 ± 0.25 a
2		2.58 ± 0.10 b	0.80 ± 0.05 bc	3.38 ± 0.14 b	0.86 ± 0.03 b	4.24 ± 0.17 b
3		2.82 ± 0.15 b	0.90 ± 0.06 ab	3.72 ± 0.20 b	0.94 ± 0.04 b	4.66 ± 0.24 b
4		3.32 ± 0.08 a	1.01 ± 0.03 b	4.33 ± 0.11 a	1.06 ± 0.02 a	5.39 ± 0.13 a
LSD	$P \leq 0.05$	0.388	0.135	0.478	0.091	0.566
	$P \leq 0.01$	0.588**	0.204**	0.727**	0.138**	0.858**

Chl—Chlorophyll; Carot—Carotenoides; *Significant at 0.05 probability level; **Significant at 0.01 probability level. Columns with different letters differ significantly at $P < 0.05$ by one-way ANOVA with Duncan's multiple range test.

Table 3. The effects of different populations of *R. serbica* from Kosovo, Albania and Macedonia on photosynthetic pigment content in mg per gram dry weight (DW).

Code of populations		Chl *a*	Chl *b*	Total Chl	Carotenoids	Total Chl + Carot
		Mean ± SX	Mean ± SX	Mean ± SX	Mean ± SX	Mean ± SX
5		3.43 ± 0.09 de	1.14 ± 0.04 d	4.57 ± 0.13 c	1.11 ± 0.03 bcd	5.68 ± 0.15 cd
6		4.38 ± 0.08 a	1.43 ± 0.05 c	5.81 ± 0.12 a	1.19 ± 0.05 ab	7.00 ± 0.15 a
7		2.86 ± 0.08 g	1.76 ± 0.06 a	4.61 ± 0.13 c	0.81 ± 0.02 f	5.43 ± 0.15 cd
8		4.47 ± 0.13 a	1.43 ± 0.04 c	5.90 ± 0.16 a	1.16 ± 0.05 bc	7.06 ± 0.20 a
9		3.70 ± 0.09 c	1.34 ± 0.03 c	5.04 ± 0.12 b	1.07 ± 0.02 cd	6.12 ± 0.14 b
10		3.49 ± 0.07 cde	1.10 ± 0.03 d	4.58 ± 0.08 c	1.08 ± 0.02 cd	5.66 ± 0.10 cd
11		3.42 ± 0.11 de	1.19 ± 0.04 d	4.62 ± 0.10 c	1.04 ± 0.05 d	5.66 ± 0.11 cd
12		3.37 ± 0.07 e	1.10 ± 0.02 d	4.46 ± 0.09 c	1.08 ± 0.02 cd	5.55 ± 0.11 cd
13		3.13 ± 0.07 f	1.20 ± 0.02 d	4.33 ± 0.09 c	1.05 ± 0.02 d	5.37 ± 0.12 d
14		4.02 ± 0.08 b	1.58 ± 0.06 b	5.61 ± 0.13 a	1.26 ± 0.03 a	6.86 ± 0.14 a
15		3.67 ± 0.08 cd	1.42 ± 0.03 c	5.09 ± 0.11 b	1.11 ± 0.03 bcd	6.20 ± 0.13 b
16		2.96 ± 0.06 fg	0.81 ± 0.02 e	3.76 ± 0.07 d	0.93 ± 0.02 e	4.69 ± 0.09 e
17		2.86 ± 0.06 g	0.84 ± 0.03 e	3.70 ± 0.09 d	0.93 ± 0.02 e	4.63 ± 0.11 e
18		3.59 ± 0.09 cde	1.10 ± 0.03 d	4.68 ± 0.12 c	1.13 ± 0.03 bcd	5.81 ± 0.15 bc
LSD	$P \leq 0.05$	0.339	0.149	0.431	0.098	0.492
	$P \leq 0.01$	0.458**	0.201**	0.582**	0.132**	0.470**

Chl—Chlorophyll; Carot—Carotenoides; *Significant at 0.05 probability level; **Significant at 0.01 probability level. Columns with different letters differ significantly at $P < 0.05$ by one-way ANOVA with Duncan's multiple range test.

spectively).

At the Gorge of Zhlebi population was observed the lowest value of Ratio Chl *a/b* and the highest the Ratio of Total Chl/Carot (5.67). Gonçalves *et al*. (2001) [16] confirm a slight differences in Chl *a*/*b* ratio and chlorophyll/carotenoid ratio between species and environments. In the open site, the Chl *a*/*b* ratio was higher and chlorophyll/carotenoid ratio was lower in tonka bean and ma-

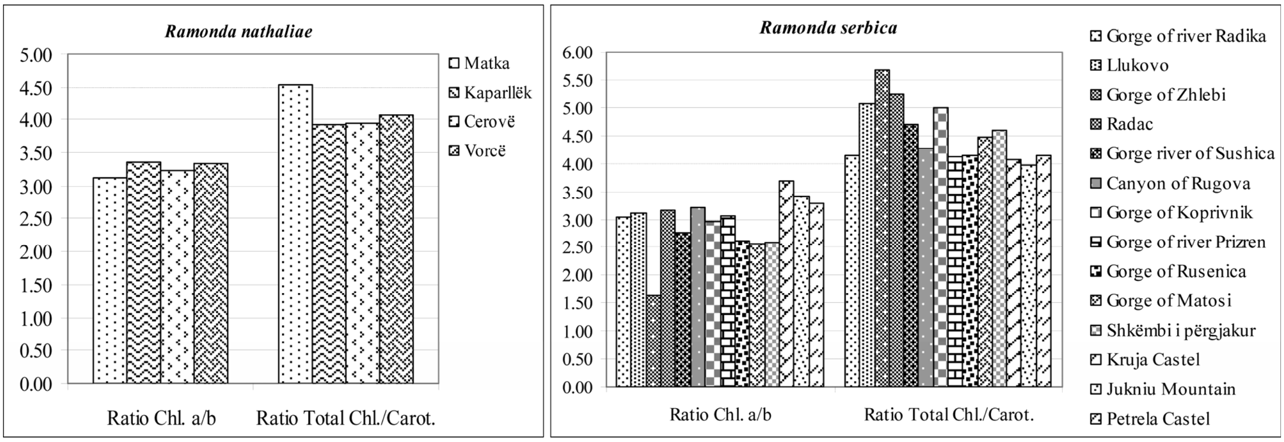

Figure 1. Ratio chlorophyll *a*/*b* and Total chlorophyll/Carotenoids of *Ramonda* plants from different populations.

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4. Conclusions

The populations with north-east direction and the highest exposition to the sunlight have lowest photosynthetic pigment content compared to populations with less sunlight. The lowest Chl *a*/*b* ratio is at populations with less sunlight whereas the highest Chl/Carot ratio.

According to statistical analysis results with Duncan test, the populations' alignment with approximate content of photosynthetic pigments clearly indicates the similar ecological conditions of these populations.

There are no significant differences of photosynthetic pigments content between populations and their elevation.

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